FEASIBILITY OF USING BIOLOGICAL DEGRADATION FOR THE ON-SITE TREATMENT OF MIXED WASTES

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ABSTRACT: This research was conducted to investigate the feasibility of applying microbial biodegradation as a treatment technology for wastes containing radioactive elements and organic solvents (mixed wastes). In this study, we focused our efforts on the treatment of wastes generated by biomedical research as the result of purifying tritium labeled compounds by high-performance liquid chromatography (HPLC). These wastes are typically 80% water with 20% acetonitrile or methanol or a mixture of both. The objective was to determine the potential of using biodegradation to treat the solvent component of tritiated mixed waste to a concentration below the land disposal restriction standard (1 mg/L for acetonitrile). Once the standard is reached, the remaining radioactive waste is no longer classified as a mixed waste and it can then be solidified and placed in a secure landfill. This investigation focused on treating a 10% acetonitrile solution, which was used as a non-radioactive surrogate for HPLC waste, in a bioreactor. The results indicated that the biodegradation process could treat this solution down to less than 1 mg/L to meet the land disposal restriction standard.

INTRODUCTION

There has been increasing interest in developing alternatives to current treatment technology for low-level mixed waste (LLMW) as tighter regulations on low-level radioactive waste (LLRW) have caused significant increase in its disposal cost (NRC, 2001). LLMW, which is composed of LLRW and hazardous waste (e.g. organic solvents), is mainly generated as a by-product of various activities associated with biomedical research, medical procedures, nuclear power plant operations, and nuclear weapons research (USEPA, 2002).

The main disposal issue is that LLMW contains both radioactive and hazardous elements. Therefore, the Land Disposal Restriction (LDR) regulations under the Resources Conservation and Recovery Act (RCRA) must first be met before the radioactive components can be solidified and placed in a secure landfill (USEPA, 2002; Chang et al., 2001). This study was conducted to investigate the feasibility in applying a biodegradation process towards treating the hazardous waste component of LLMW. We focused our efforts on the treatment of a biomedical research waste generated during the purification of tritium labeled compounds by high-performance liquid chromatography (HPLC).

The HPLC mixed waste generally contains 40-90% water and 10-40% organic content (usually dominated by acetonitrile and methanol), along with relatively low radioactivity. Bacterial culture were developed that could grow on methanol and acetonitrile at high concentrations (Chang et al., 2001). The degradation of the main HPLC solvents, acetonitrile and methanol, by this culture had been found to follow well-

known kinetic models (Monod or Haldane) during a bench-scale growth and substrate oxidation kinetics measurements (Chang et al., 2001). This indicated that scale-up of the biodegradation process to pilot or full-scale treatment can follow standard engineering protocols. Furthermore, acetonitrile was found to be an inhibitory substrate that was more difficult to degrade than methanol (Chang et al., 2001).

This study examined the biological treatment of a 10% acetonitrile solution as the surrogate HPLC mixed waste. This solution was continuously fed at varying rates to a semi-batch, continuously-stirred bioreactor containing the previously developed bacterial culture. The acetonitrile, biomass, and ammonia concentrations were monitored throughout this investigation. Our goal was to achieve less than 1 mg/L acetonitrile concentration in the reactor to meet the LDR standards, without resorting to excessive dilution of the waste stream.

MATERIALS AND METHODS

Materials. The acetonitrile enrichment culture used in this study was developed as described by Chang et al. (2001). 10 mL of the acetonitrile enrichment culture was inoculated into one liter of nitrogen-free minimal media (NFMM). Two sets of this culture were prepared (total of two liters). The acetonitrile concentrations in these cultures were kept between 100 and 200 mg/L. After 17 days of this maintenance, 200 mL of NFMM was added to both cultures to make up the total volume of 1.2 liters per culture. 300 mL draw-fill maintenance was done on these cultures every four days.

The NFMM consisted of 1000 mg/L KH_2PO_4, 860 mg/L Na_2HPO_4, 120 mg/L $MgSO_4$, 60 mg/L $CaCl_2$, and 1 mL/L of trace metal solution. The trace metal solution was composed of 3.3 mg/L $MnSO_4$-H_2O, 6.2 mg/L $CuSO_4$-$5H_2O$, 7.6 mg/L $ZnSO_4$-$7H_2O$, 11.7 mg/L Na_2MoO_4-$2H_2O$, 64.6 mg/L $FeSO_4$-$7H_2O$, and 4.15 mL/L HCl.

Methods. The acetonitrile biotreatment test was conducted in a New Brunswick Bioflo 3000 bioreactor. The reactor was filled with two liters of bacterial culture suspended in NFMM. Oxygen flow to the reactor ranged between 4 and 5 mL/min and an agitation speed of 150 rpm was employed. The temperature of the reactor was maintained at 25°C. Before being inoculated into the reactor, the acetonitrile enrichment culture was washed three times with NFMM. The washing was done by centrifuging the enrichment culture (total volume of 2.4 liters) with Beckman J2-HS centrifuge at 8000 rpm for 10 minutes per wash. JLA 10.500 rotor was used for the centrifugation. This rotor held six 500-mL capacity containers. 400 mL of the enrichment culture was dispensed into each container for the first wash. The culture pellet was resuspended in each container with NFMM, and then combined into two of the six 500-mL containers. The volume of resuspension for each container was 250 mL. The second washing was done by centrifuging these two containers. The culture pellets were resuspended again with 250 mL of NFMM for each container, and then centrifuged again for the third wash. The pellets were resuspended with 50 mL of NFMM for each container, and were inoculated directly into the bioreactor.

The 10% (by weight) acetonitrile influent was fed to the reactor with Ismatec IPC (16-channel, 8-roller) tubing pump at the rates ranging between two and six mL/day using Masterflex Norprene Food tubing, size L/S 16 (Cole-Parmer Instruments, Vernon

Hills, Illinois). The flow was commenced immediately after the enrichment culture inoculation. The initial influent flow was set to 4 mL/day.

The reactor sampling was done in order to conduct acetonitrile, optical density (for biomass concentration), and ammonia analyses. Samples were drawn out from a sampling port (screw cap port on top of the reactor vessel) with 10-mL disposable glass pipettes into 13-mm culture tubes. NFMM was added after each sampling so that the volume in the reactor was kept constant at two liters.

The optical density (OD) of reactor sample was obtained by reading its absorbance at 590 nm on HACH DR/2000 Direct Reading Spectrophotometer. 10 mL of reactor sample was transferred into a 13-mm culture tube, and the readings were taken by directly inserting the culture tube into the spectrophotometer. OD was converted to mg/L biomass using a conversion factor determined from dry weight measurements.

For the ammonia analysis, a sample from the reactor was diluted to 1/250 (2 mL in 500 mL) for up to Day 2, 1/500 (1 mL in 500 mL) for Day 3 to Day 11, 1/1000 (1 mL in 1000 mL) for Day 12 to Day 97, 1/2000 (1 mL in 2000 mL) for Day 98 to Day 138, and 1/4000 (0.5 mL in 2000 mL) for Day 138 until the end of this study. The ammonia reading was done on the diluted samples by the HACH Nessler method (Method 8038, HACH Water Analysis Handbook, 3rd Edition, p825). Perkin Elmer UV/VIS Spectrometer Lambda 20 was used to read the absorbance of samples (from HACH Nessler method) at the wavelength of 425 nm.

The acetonitrile analysis of the reactor and influent was done using HP6890 gas chromatograph fitted with a flame ionization detector (FID). 5 mL of reactor sample was first dispensed into a 13-mm culture tube. This sample was acidified with two drops of 6N hydrochloric acid solution. 1.6 mL of the acidified culture was dispensed into a centrifuge tube and centrifuged at a speed of 9100 rpm for 3.5 minutes. 1.5 mL of supernatant phase was then transferred into a 2-mL Kimble Chromatography Robovial for acetonitrile GC analysis. Triplicate samples were analyzed.

RESULTS AND DISCUSSION

This study was conducted over 250 days during which the reactor was continuously operated. The acetonitrile (theoretical and actual) and biomass concentrations in the reactor for the entire study duration are shown in Figure 1 and Figure 2, respectively. Figure 3 is the plot of the data from Day 34 through Day 75 for acetonitrile concentration in the reactor with respect to pH adjustment.

Figure 1 shows the comparison between the theoretical and actual acetonitrile concentrations in the reactor. The theoretical concentration indicates what the acetonitrile concentration would have been in the reactor if there were no biodegradation. The actual concentration in the figure is the actual concentration measured by GC. The difference between these two concentrations represents the amount of biodegradation. In a separate physical loss experiment, it was found that the physical loss of acetonitrile from the reactor was not significant. The flat regions on the theoretical plot are the days when the influent feed was shutoff. On the 250th day, the actual acetonitrile concentration in the reactor was <1 mg/L, which met the LDR standards, and the theoretical concentration was 25,855 mg/L. Therefore, approximately 52 g of acetonitrile was biodegraded during this study.
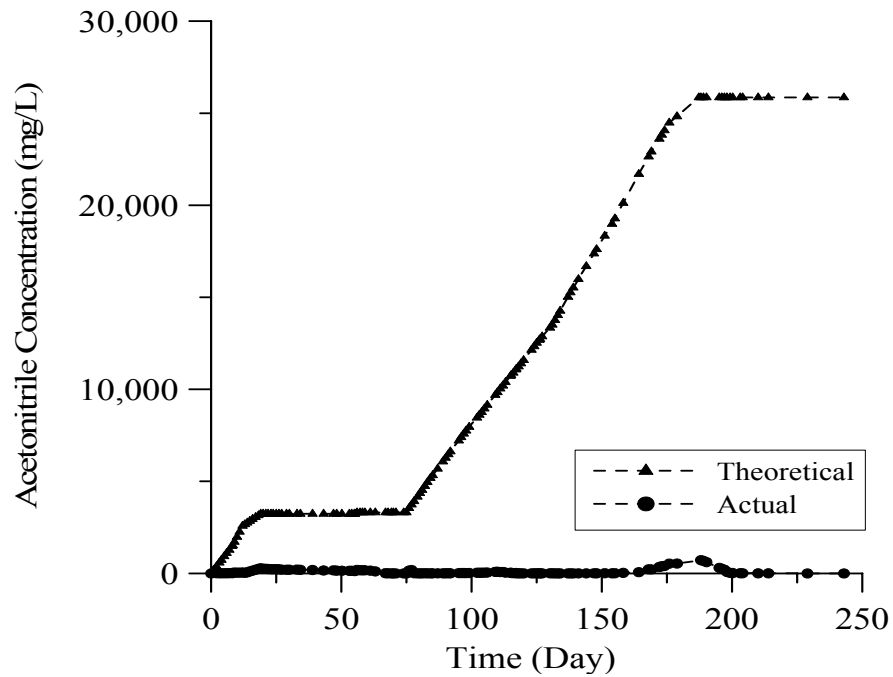
**FIGURE 1. Actual and theoretical acetonitrile concentrations in
the reactor. The actual acetonitrile concentration values with
respect to the theoretical values indicate effective biodegradation.**

Figure 2 shows the interesting biomass growth pattern in the reactor. There was a
steady biomass growth for the first 10 days. The biomass stopped increasing from Day
12 forward and the acetonitrile concentration in the reactor started to increase, indicating
that biological treatment had ceased. The biomass and acetonitrile concentration data in
Figures 2 and 3, respectively, indicate that there was little if any biodegradation in the
reactor from Day 12 until Day 59. The influent feed was halted on Day 19 as the
acetonitrile concentration had reached 280 mg/L. By Day 54, the acetonitrile had only
gone down to 146 mg/L.

Between Day 19 and Day 54, we investigated whether ammonia inhibition was
responsible for the loss of biomass activity. It was found that the ammonia concentration
in the reactor ranged between 723 mg/L and 808 mg/L during Day 12 to Day 54. This is
not an unusually high ammonia concentration, therefore ammonia inhibition was ruled
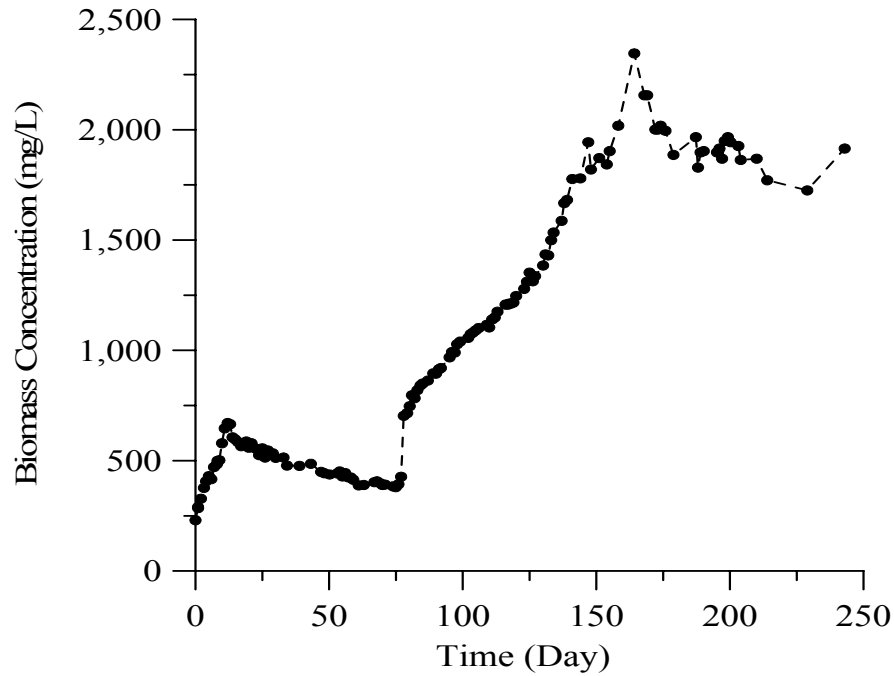out.

FIGURE 2. The biomass in the reactor grew while the acetonitrile was effectively biodegraded. When the biomass activity slowed, the acetonitrile concentration in the reactor increased accordingly.
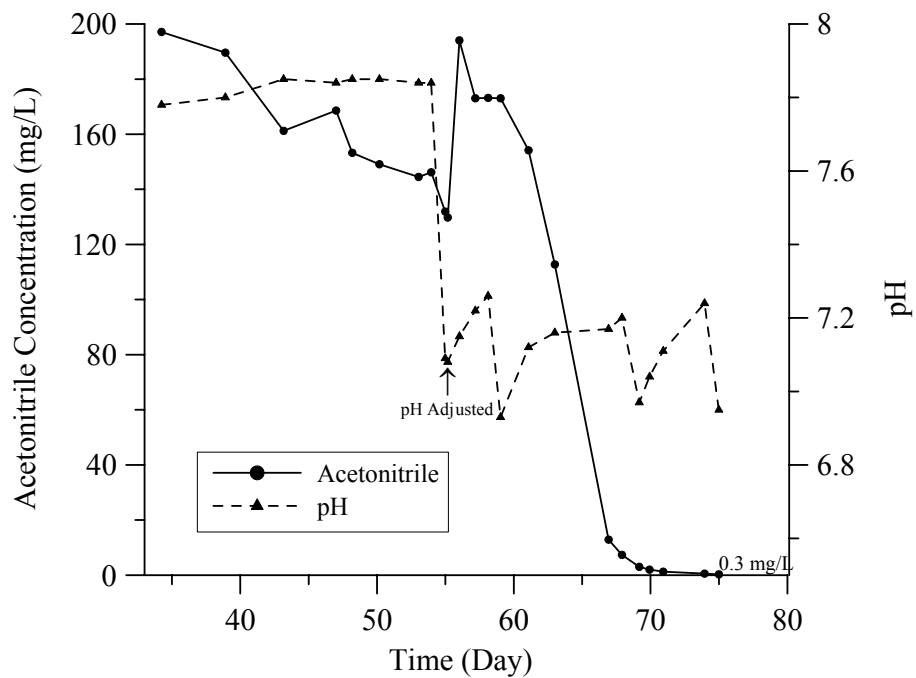

FIGURE 3. Acetonitrile concentrations and pH level in the reactor. This is the plot of data from Day 34 through Day 75, indicating the pH sensitivity of acetonitrile biodegradation.

We next tested the pH sensitivity of the acetonitrile biodegradation reaction. The production of ammonia from acetonitrile degradation had raised the reactor pH from 6.9 to 7.5 between Day 10 and Day 17. By Day 54 the pH had reached 7.84. Although the pH was still well within accepted limits for biological treatment, we adjusted the pH downward commencing on Day 54. We periodically added 6N hydrochloric acid solution to the reactor to maintain the pH at approximately 7.1. Almost immediately, as the pH was adjusted, the acetonitrile concentration in the reactor started to decrease dramatically. Figure 3 clearly depicts this concentration decrease. The acetonitrile concentration in the reactor decreased from 194 mg/L on Day 56 (the concentration had increased from 146 mg/L on Day 54 because the influent feed was inadvertently turned on from Day 55 to Day 56), to 12.9 mg/L on Day 67. By Day 75, the acetonitrile concentration had dropped well below 1 mg/L. These data show that the biodegradation reaction is very pH sensitive.

The biomass concentration did not increase again until Day 75 when the influent feed was turned back on. As indicated on Figure 2, the biomass concentration jumped from 380 mg/L on Day 75 to 703 mg/L on Day 78. From Day 75, the biomass concentration constantly increased while the acetonitrile was effectively degraded. The biomass growth continued until Day 164 when it reached a concentration of 2,346 mg/L. The influent was shutoff on Day 187 because the acetonitrile concentration in the reactor had reached 746 mg/L, and it was apparent that the degradation rate was not keeping up with the influent feed rate. At this time, the feed was shutoff and the reaction was allowed to go to completion. The acetonitrile concentration eventually reached below 1 mg/L on Day 229. By Day 195, the ammonia concentration had exceeded 6,000 mg/L in the reactor. Therefore by this time, the biodegradation activity was most likely slowed due to ammonia inhibition.

CONCLUSIONS

The main objective of this investigation was achieved, as the acetonitrile was biodegraded in the reactor to the final concentration of less than 1 mg/L. The residual material had reached the LDR standard, and thus could be disposed of in a landfill. It was found in this study that the acetonitrile degradation reaction was pH sensitive, where the optimal pH range for the biodegradation process was approximately between 6.5 and 7.1. Above pH 7.5 the reaction was severely inhibited. The physiological reason for this inhibition is not known at this time.

Data from the theoretical acetonitrile concentration plot in Figure 1 indicated that the final acetonitrile concentration in the reactor would have been 25,855 mg/L if there were no biodegradation in the reactor. This meant that the treatment had only required an approximately 4-fold volume dilution of the original material, which is an acceptable volume change for this waste stream. These results indicate that the development of a biodegradation process for a mixed solvent and radioactive waste streams is entirely feasible.

ACKNOWLEDGMENTS

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